Rabbit Syncytium Virus Is a Kemerovo Serogroup Orbivirus†

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Polyacrylamide gel genome electropherotyping and negative-stain electron microscopic studies, along with immunofluorescent staining and immune electron microscopy reactions, indicate that rabbit syncytium virus has the morphologic, genomic, and antigenic attributes of a Kemerovo serogroup orbivirus.

Rotaviruses cause diarrhea in humans and other animals (7), and at least five distinct rotavirus serogroups, designated A through E, exist (18, 19). The discovery that trypsin permits group A rotavirus cell culture propagation (26) has allowed extensive characterization of isolates belonging to this serogroup. However, techniques for routine serial propagation of non-group A rotaviruses in cell culture are lacking, and investigations on these viruses are seriously restricted. Consequently, cultivatable non-group A rotaviruses are of considerable medical and veterinary importance.

Rabbit syncytium virus, isolated from a healthy cottontail rabbit (*Sylvilagus floridanus*) trapped in Quinby, Va., in 1962, induces syncytia in some cell cultures (16). This virus most closely resembled the EDIM agent (a group A rotavirus) in morphology and morphogenesis, but was antigenically unrelated to it (4, 16). Syncytial cell formation, however, often occurs during group B rotaviral infections in vivo and in vitro (30, 32, 33). Therefore, we investigated the possibility that rabbit syncytium virus might be a cultivatable group B rotavirus.

Amniotic-allantoic fluid from the 12th egg passage of rabbit syncytium virus (10 January, 1963) recovered from rabbit 32 and the 5th egg passage of its reisolation from rabbit 32 tissues (29 August, 1964) was kindly provided by M. F. Bozeman, Food and Drug Administration, Department of Health & Human Services, Bethesda, Md. Roller tube monolayers of MA104 and BHK-21 cells, prepared as described previously (28) but without trypsin in the maintenance medium, were inoculated with egg-passaged virus diluted 10-fold. Rabbit syncytium virus was recovered from each egg passage and was further passaged in both cells as described above, although only BHK-21 cells were used for the later passages. Virus passages were stored at 4 or –20°C in 50% glycerol.

Rabbit syncytium virus induced a cytopathic effect in both cell lines which was characterized by foci of rounded cells that gradually enlarged to destroy the monolayer. Some MA104 cell monolayers, prepared on coverslips as described previously (30), were infected with rabbit syncytium virus and subsequently stained with Harris hematoxylin and eosin after fixation in Bouin solution. Stained infected monolayers contained many cells with eosinophilic and basophilic cytoplasmic inclusion bodies, but no syncytia (Fig. 1). Other infected MA104 cell coverslip monolayers were examined by immunofluorescence microscopy, using fluorescein-conju-

gated antibodies to bovine group A rotavirus (30) and porcine group C rotavirus (2), or by indirect immunofluorescence microscopy, using gnotobiotic pig group B rotavirus (31) and reovirus type 3 (2) antisera. Rabbit syncytium virus-infected cells did not contain group A rotaviral or reoviral antigens, confirming previous results (4, 16), or group B or C rotaviral antigens (Fig. 2).

Negatively stained lysates of rabbit syncytium virus-infected cells, prepared by the method of Lecatsas and Gorman (14), were examined by electron microscopy (29) and found to contain spherical virus particles of about 60 nm in diameter (Fig. 3). An amorphous outer layer often covered these virions and obscured their internal structure. In contrast, negative staining of rabbit syncytium virus concentrated by ultracentrifugation from frozen and thawed infected cells revealed virions lacking an outer layer and with ringlike structures clearly evident (Fig. 3); such virions were similar to those described earlier (4) and resembled single-shelled rotavirus particles. Hyperimmune gnotobiotic pig antiserum to cell culture-passaged rabbit syncytium virus,

FIG. 1. Cytopathic effect of rabbit syncytium virus in MA104 cells. (a) Noninoculated control monolayer. (b) Infected monolayer, 48 h postinoculation. Numerous basophilic and eosinophilic cytoplasmic inclusion bodies are apparent. Harris hematoxylin and eosin stain was used. Magnification, $\times 400$.

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FIG. 2. Indirect immunofluorescent staining of BHK-21 cell monolayers, using hyperimmune gnotobiotic pig antiserum to rabbit syncytium virus. Monolayers were infected with (a) rabbit syncytium virus; (c) bluetongue virus (Bluetongue serogroup); (d) Corripa virus (Corripa serogroup); (e) Irituia virus (Changuinola serogroup); or (f) Tribec virus (Kemerovo serogroup). Specific immunofluorescence is characterized as bright intracytoplasmic granules in the rabbit syncytium and Tribec virus-infected monolayers. (b) Also shown is indirect immunofluorescent stain of a BHK-21 monolayer infected with rabbit syncytium virus, using hyperimmune gnotobiotic pig antiserum to porcine group B rotavirus (Ohio isolate). Magnification, $\times 200$.

prepared as described for group A rotavirus antiserum (28), was used in immune electron microscopy studies. Diluted antiserum was added to suspensions containing rabbit syncytium virus, reovirus (2), group A rotavirus (28), group B rotavirus (31), group C rotavirus (2) or group D rotavirus (20, 29), and the mixtures were examined for clumped virus as described previously (29); only homologous virus was clumped by this antiserum (Fig. 3).

Viral double-stranded RNA was extracted from infected cell lysates by CF11 cellulose chromatography (27), separated in a Laemmli 7.5% polyacrylamide slab gel, and stained with silver as described previously (3). The rabbit syncytium virus genome comprised 10 double-stranded RNA segments that produced an electropherotype distinct

FIG. 3. Electron microscopy and immune electron microscopy of rabbit syncytium virus and its antiserum. (a) Negative stain of infected BHK-21 cell lysate showing virions coated with an amorphous outer layer. (b) Negative stain of virus from frozen and thawed MA104 cells showing a virion devoid of the outer layer. Immune electron microscopic reactions, using hyperimmune gnotobiotic pig antiserum to rabbit syncytium virus, reacted with (c) rabbit syncytium virus; (d) bovine group A rotavirus (NCDV isolate); (e) group C rotavirus (Cowden isolate); (f) turkey group D rotavirus; (g) reovirus type 3 (Abney isolate). Bar, approximately 70 nm.

from those produced by the reovirus and group A rotavirus genomes (Fig. 4). These segments, with molecular weights ranging from about 2.5×10^6 to 0.4×10^6 , were distributed in a pattern similar to that of the genome electropherotypes of orbiviruses belonging to the Corripa and Kemerovo serogroups (9, 10, 17): 2 large, 4 intermediate, 3 medium, and 1 small.

Four orbiviruses, bluetongue virus serotype 8 (Bluetongue serogroup), Irituia virus (Changuinola serogroup), Corripa virus (Corripa serogroup), and Tribec virus (Kemerovo serogroup), from the American Type Culture Collection, Rockville, Md., were passaged in BHK-21 cells in roller tubes and then inoculated onto BHK-21 cell coverslip monolayers as described above. At 48 h postinoculation, monolayers were fixed in acetone and examined by indirect immunofluorescence microscopy, using hyperimmune anti-rabbit syncytium virus serum and fluorescein-conjugated anti-porcine immunoglobulin G antibody (ICN Biomedicals Inc., Costa Mesa, Calif.). Immunofluorescence was observed only with cells infected with rabbit syncytium or Tribec (Fig. 2) virus and consisted of small, discrete, brightly fluorescent, intracytoplasmic granules. In addition, cells infected with rabbit syncytium or bluetongue virus were examined by indirect immunofluorescence microscopy, us-

FIG. 4. Comparison of reovirus type 3, Abney isolate (A), rabbit syncytium virus (B), and equine group A rotavirus, FI-14 isolate (C), genome electropherotypes in the same polyacrylamide gel slab. Migration is from top to bottom. Numbers to the right of each lane designate segment numbers.

ing hyperimmune bovine anti-bluetongue virus serum, kindly provided by the National Veterinary Services Laboratories, Ames, Iowa, and fluorescein-conjugated anti-bovine immunoglobulin G antibody (ICN Biomedicals); immunofluorescence was observed only with cells infected with bluetongue virus.

Our findings demonstrate that rabbit syncytium virus is not a cultivatable non-group A rotavirus, but it possesses the morphologic, genomic, and antigenic attributes of a Kemerovo serogroup orbivirus. Kemerovo serogroup orbiviruses are tickborne (11), and some have been associated with human encephalitis in Eurasia (6, 15). Until now, however, Kemerovo serogroup orbiviruses have been reported in the United States only from ixodid or argasid ticks infesting the restricted habitats of sea bird or cliff swallow nesting sites (5, 21, 34, 35). In these environments, opportunities for virus transmission to humans are limited. On the other hand, cottontail rabbits and the ticks commonly infesting them (1, 8, 22, 24, 25) are widely distributed in North America, thus providing more opportunities for virus transmission to other hosts. Two of these ticks, *Haemaphysalis leporispalustris* and *Ixodes dentatus*, rarely bite humans (1), but a third, *Amblyomma americanum*, does and is prevalent throughout the southeastern United States.

Little is known regarding the natural history of rabbit syncytium virus, although limited serologic data indicate that infections are restricted to cottontail rabbits and negative serologic findings suggest that humans are not infected (16). Nonetheless, collection of additional data on this potential human pathogen would be prudent, as alterations in human activities and the environment can change patterns of tickborne diseases (12, 13); the recent emergence of human babesiosis and Lyme disease provides two instructive examples on how such changes have led to conditions favorable to the spread of tickborne disease (23). The distribution of rabbit syncytium virus in the cottontail rabbit population and the vector(s) responsible for virus transmission remain to be determined.

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